

## **REMARKS**

The Office Action of January 16, 2002 has been received and carefully reviewed, and the substitute specification along with the following comments are a complete response thereto.

Claims 1-23, 25 and 26 are all the claims under examination. By this Amendment, Claims 1-23, 25 and 26 have been amended as discussed below.

### **I. Withdrawal of Restriction Requirement**

Applicants gratefully acknowledge the Examiner's withdrawal of the restriction requirement of the claims based on the arguments filed in the response of November 2, 2001. Accordingly, claims 16 and 17 have been rejoined with claims 1-15, 18-23 and 25 and 26 for consideration.

### **II. Response to Objection to the Specification and Claim 19**

**A.** The Examiner objects to the specification because it lacks division into distinct sections as required.

A substitute specification is attached hereto and which complies with the specification formatting requirements.

**B.** Claim 19 is objected to because it does not end with a period.

Claim 19 has been amended to insert a period as appropriate. This amendment does not effect the scope of the claim.

**III. Response to Rejection of Claims 1-23, 25 and 26 under 35 USC §112, second paragraph**

Claims 1-23, 25 and 26 are rejected under 35 USC §112, second paragraph, for indefiniteness.

**A.** According to the Examiner, Claim 13 is indefinite for reciting “chemical integrity”.

Example 3 in the specification explains in detail the measurements performed in order to characterize the chemical “stability” of the formulation over long term. Accordingly, the claim has been amended to replace the term “integrity” with “stability”, even though “chemical integrity” is just as acceptable based on the definition provided on page 9 at lines 31-37. This amendment does not effect the scope of the claim.

**B.** According to the Examiner, Claims 15 and 26 are indefinite for reciting “physical integrity”.

Contrary to the Examiner’s assertion, Claim 15 does not recite the phrase.

Applicants cite page 10 at lines 3-9 of the specification in support of the use of the phrase “physical integrity”, and submit that the phrase is definite with respect to Claim 26.

**C.** According to the Examiner, Claims 18 and 26 are indefinite for reciting “auxiliaries”.

“Auxiliary” substances are defined on page 10, line 36- page 11, line 12 of the specification. However, this general class of substances can also be referred to as “ingredients”, and in amending Claim 18 to recite this term, Applicants are not introducing new matter or altering the claim scope.

Claim 26 does not recite “auxiliaries”, contrary to the Examiner’s assertion.

D. According to the Examiner, Claims 1-3 are indefinite for reciting “as active ingredient” since the claims are missing the article (“an” or “the”), and which is the biological activity being referred to if the article “an” is present. Additionally, the standard terminology for representation of units of IFN- $\beta$  is  $25 \times 10^6$  U/ml instead of 25 MU/ml as recited in claim 1.

Applicants submit that an active ingredient is more typically found in a pharmaceutical composition claim. In having amended claims 1-3 to recite a “liquid pharmaceutical formulation”, the formulation containing an active ingredient finds proper basis.

Insofar as the Examiner’s rejection of the active units claimed in claim 1, Applicants have adopted the Examiner’s suggestion for bringing the claims into compliance under U.S. practice, i.e., reciting “ $25 \times 10^6$  U” instead of 25 MU. This amendment does not effect the scope of the claim.

E. According to the Examiner, Claim 20 is indefinite for reciting “physiologically acceptable preservatives”.

“Physiologically acceptable preservatives” are taught on page 11 at lines 18-21, and therefore, the phrase finds support in the specification. Applicants amendment of the claim to recite “at least one physiologically acceptable preservative” does not raise an issue of new matter nor change the scope of the claim.

F. According to the Examiner, Claim 26 is indefinite for reciting “or/and” because in each case the limitations that follow the use of the phrase “or/and” are not clear.

Applicants have amended the claim to replace “and/or” language with the phrase “at least one of”, since the Examples in the specification support the three aspects of

the formulation that are stabilized, namely, the biological, chemical and physical properties.

**G.** According to the Examiner, Claim 5 is indefinite for reciting “originates from CHO cells”, since it is not clear how human IFN-  $\beta$  originates from CHO cells. The Examiner indicates that this rejection can be obviated by reciting “IFN-  $\beta$  recombinantly produced in CHO cells”.

Support for the Examiner’s suggested phrase is found on page 9 at line 10, and claim 5 has been amended accordingly. This change does not alter the scope of the claim.

**H.** According to the Examiner, the recitation in claim 6 of “a concentration of 10 mM/l to 1 mol/l” is indefinite because it is not clear as to what is the buffer constituent, such as “phosphate or acetate” that is present at the said concentration.

Applicants disagree that the claim should recite an embodiment for any one type of buffer in order for to claim a concentration range for a “buffer”. Applicants are not aware of any rule or case law, which precludes claiming a concentration range for a generic buffer.

**I.** According to the Examiner, Claim 23 is indefinite because it is not clear if recitation of “unit doses” is meant as an additional element, or is it intended as “packaged in unit doses of  $1-25 \times 10^6$  IU”.

Applicants submit that the pharmaceutical composition would be in the form of a unit dose containing contain  $1-25 \times 10^6$  IU of INF- $\beta$ , and claim 23 has been amended to

more clearly recite the intended subject matter. This amendment does not effect the scope of the claim.

J. According to the Examiner, Claim 25 is indefinite because the only required step is a negative limitation and involves no process steps. Therefore, the claim is incomplete. Additionally, "using a formulation" is not "a process for improving the shelf life" and use of the term "using" is indefinite because it is not a method step.

Claim 25 has been amended to recite process steps for stabilizing the formulation.

K. According to the Examiner, the use of parenthesis in claim language make claim 26 indefinite because it is not clear if the parenthetical phrase "is" or "is not" a limitation.

The Examiner appears to have examined original claim 26 rather than the amended claim included on the amended pages of the national stage application. Amended claim 26 has already been corrected in this aspect, and no further action need be taken on this matter.

#### **IV. Response to Rejection to Claims 1-17, 21-23, 25 and 26 under 35 USC §103(a)**

Claims 1-17, 21-23, 25 and 26 are rejected under 35 USC §103(a) as being obvious over U.S. Patent No. 5,151,265 (1992) in view of U.S. Patent No. 5,358,708 (10/25/1994).

According to the Examiner, '265 teaches a liquid pharmaceutical composition of non-lyophilized IFN- $\gamma$ , or recombinant IFN- $\gamma$  including a buffer capable of maintaining the pH of the liquid composition within the pH range of 4.0 - 6.0 (abstract, lines 1-6).

The formulation contained  $5 \times 10^6$  U/ml (column 3, Example 1, lines 1-2, column 4, 2nd paragraph, lines 1-2) of the cytokine, and was stable for prolonged periods when stored at -20 °C to 30 °C with the preferred storage temperature in the range of 2-8 °C (column 3, entire 4th paragraph), in succinate buffer and mannitol as the stabilizing agents (claim 15). '265 also disclosed that the shelf life was better in the absence of human serum albumin (Table 1, column 4), thus meeting all of the limitations of instant claim 1. '265 also teaches liquid pharmaceutical compositions of recombinant IFN- $\gamma$ , which meets the limitations of instant claims 21-23. However, '265 is silent as to INF- $\beta$ .

'708 teaches several approaches taken to stabilize and prepare protein formulations of IFN- $\alpha$ , erythropoietin, human plasminogen, IL-2, and plasmin (column 1, paragraph 3-7). In particular, disclosure of '708 also points out that their invention can be used "to stabilize all of the proteins in the IFN family produced in the human body, including related, or recombinant proteins, which confer resistance to viral infection, affect proliferation of cells and modulate the response of the immune system" including IFN- $\beta$  (column 2, detailed description of the invention, lines 44-58).

In combining the disclosures of '265 and '708, the Examiner considers the claims *prima facie* obvious.

Applicants traverse and submit that the Examiner's rejection based on the combined reference disclosures fails to satisfy a *prima facie* case of obviousness since the references alone or in combination, do not teach or suggest the invention as a whole (MPEP §2141.02) or provide objective reasons for modifying their disclosures to obtain the inventive processes and kit (MPEP §2143.01).

Claims 1-17, 21-23, 25 and 26 are directed to a liquid pharmaceutical formulation, a pharmaceutical composition or a process for making the same where the formulation and composition have **long-term stability at room temperature** and they contain **human interferon- $\beta$**  as an active ingredient, a buffer in a **physiological pH** range, and human serum albumin or any acidic amino acids, arginine or glycine in amounts of between 0.3 and 5% by weight are excluded.

'265 only teaches long-term stabilization of **INF- $\gamma$** -containing formulations when maintained at between **-20°C to 8°C** over several months. The reference does not teach or suggest the effects on stability when liquid formulations containing any other cytokine but INF- $\gamma$  are held at 25° over several months.

In the Examples of '265, the biological activity and physical stability of its INF- $\gamma$  formulations were measured after long-term storage at 5°C. '265 does not teach or suggest the importance of stabilizing biological activity, chemical stability **and** physical stability of its formulations at room temperature for prolonged periods of time. Thus, it is not apparent that preserving shelf-life for a liquid INF- $\beta$  formulation would be inherent to '265.

The '265 patent does not teach or suggest, as the Examiner well appreciates, that a formulation in the absence of human serum albumin would have a higher stability than in the presence thereof. Applicants direct the Examiner's attention to Table 1 of the patent, where two different lyophilizates are compared, i.e., succinate/mannitol and HAS/phosphate. The lyophilized HAS solution has a higher stability, and while it is a fact that a liquid succinate/mannitol solution is more stable than the HAS/phosphate

lyophilizate, no comparison is made for a liquid HAS/phosphate solution.<sup>1</sup> Accordingly, the '265 patent does not provide any evidentiary support for the Examiner's assertion that the absence of HAS would result in increased stability.

Finally, Applicants submit that the pH-value for the formulations according to '265 fall within a range of between **4.0 and 6.0**, whereas according to the present invention, a physiological pH value is preferred.

The '265 patent does not teach or suggest IFN- $\beta$ , and furthermore because different proteins each have specifically different requirements for stabilizing their physical and chemical integrity, one skilled in the art would not have been motivated to rely on the patent disclosure to solve the technical problem underlying the present invention.

The specification discusses the conditions under which long-term stability of cytokine formulations was ascertained in the '708 patent (page 5, lines 20-25). In the Examples of '708, chemical stability was measured after a 2-week exposure to a temperature of 40°C. Chemical stability of the formulation was tested by HPLC analysis of degradation products, but significantly, no biological activity or physical stability measurements were made.

Even assuming that '708 makes a very general disclosure for INF formulations, the reference does not teach or suggest that an INF- $\beta$  formulation would be stable over a 2-week period at 40°C, much less at any relevant temperature below 40°C (i.e., physiological temperature (37°C) or room temperature (25°C) for a period over 2 weeks)

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<sup>1</sup> The '265 patent does not make a direct comparison between a liquid HAS solution and a liquid HAS-free solution.

in the disclosed liquid formulation. The Examples in the '708 patent only refer to **interferon- $\alpha$ -2b**. This product is **not glycosylated** in contrast to the IFN- $\beta$  derived from CHO cells of the present invention.

The storage conditions of '708 do not even approximate those of '265 much less those of the claimed invention. Moreover, '708 specifically teaches that even for its own disclosed formulation, **preservation of any protein is relatively unpredictable** (or is at least empirical):

“...different proteins will become inactivated during storage at different rates and under different conditions, due to chemical differences between the proteins. The presence of trace impurities in the formulations may catalyze decomposition reactions. Also, the storage-prolonging effects of methionine and histidine are not equivalent with the different proteins, and of course, mixtures of the amino acids will exhibit different effects as the ratio is varied, the identity of the protein is changed and/or the concentrations are altered.”

(Col. 4, lines 8-18).

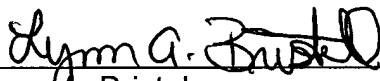
'708 teaches that the half-life of any protein in its disclosed liquid formulation is highly dependent on the species of protein, and '265 only teaches a formulation for INF- $\gamma$ . One skilled in the art would not have been motivated to combine the references in the manner asserted by the examiner, because '708 patent does not teach with any degree of surety the conditions for stabilizing an INF- $\beta$  protein much less that an INF- $\beta$  protein could be combined with the formulation of '265, to obtain an INF- $\beta$  formulation with long-term stability. Accordingly, withdrawal of this rejection is deemed proper.

## CONCLUSION

In view of the foregoing amended claims, the substitute specification and Applicants' arguments for patentability of the claims, Applicants submit that the Examiner's objection to the specification and rejection of the claims under 35 U.S.C. §§103(a) and 112, second paragraph, have been met and overcome. The Examiner is requested to allow the application to pass to issuance.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300 under Client Matter No. 100564-00002.

Respectfully submitted,

  
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**MARKED-UP COPY OF THE CLAIMS FOR 09/508,510**

1. (Amended) [Liquid] A liquid pharmaceutical formulation [which comprises] comprising human interferon- $\beta$  as an active ingredient in a concentration of up to [25 MU/ml]  $25 \times 10^6$  u/ml and a buffer for [setting] buffering in a pH range of 5 to 8, [is free from human serum albumin and after storage for 3 months at 25°C shows a long-term stability of the biological in vitro activity of at least 80% of the initial activity,] with the proviso that the formulation does not [comprise] contain human serum albumin, or any acidic amino acids, arginine or glycine in amounts of between 0.3 and 5% by weight, wherein after storage for 3 months at 25°C, stability of in vitro biological activity of the formulation is at least 80% of an initial biological activity.

2. (Amended) [Liquid] The liquid formulation according to Claim 1, [characterized in that it comprises] comprising a buffer for [setting] buffering in a pH range of 6 to 7.2.

3. (Amended) [Liquid] A liquid formulation [which comprises] comprising human interferon- $\beta$  as the active ingredient, a buffer for [setting] buffering a pH in a range of 5 to 8, and at least one [or more] amino [acids and shows after storage for 3 months at 25°C a long-term stability of the biological in vitro activity of at least 80% of the initial activity] acid, with the proviso that the formulation does not comprise any acidic amino acids, arginine or glycine in amounts of between 0.3 and 5% by weight, wherein after storage for 3 months at 25°C, stability of an in vitro biological activity of the formulation is at least 80% of an initial biological activity.

4. (Amended) [Formulation] The formulation according to Claim 1, [characterized in that it comprises] wherein the interferon- $\beta$  is a glycosylated interferon- $\beta$ .

5. (Amended) [Formulation] The formulation according to Claim 2, [characterized in that] wherein the interferon- $\beta$  [originates from] is recombinantly produced in CHO cells.

6. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it comprises] wherein the buffer is in a concentration of 10 mmol/l to 1 mol/l.

7. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it comprises a] wherein the buffer is selected from the group consisting of a phosphate, a citrate and an acetate [buffers and mixtures of these] buffer, and a combination thereof.

8. (Amended) [Formulation] The formulation according to Claim 7, [characterized in that it] wherein the buffer comprises a phosphate/citrate buffer.

9. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it has a] wherein the pH is between 6 and 7.2.

10. (Amended) [Formulation] The formulation according to Claim 3, [characterized in that it is free from] wherein the formulation does not contain human serum albumin.

11. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that, apart from] wherein the active ingredient[, it] is free from human or animal polypeptides.

12. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it] wherein the formulation is free from surfactants.

13. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it exhibits a chemical integrity] wherein after storage of the formulation for 6 months at 25°C, the formulation is chemically stable.

14. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it exhibits a physical integrity] wherein after storage of the formulation for 6 months at 25°C, the formulation is physically stable.

15. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it furthermore comprises] further containing at least one [or more] amino [acids] acid.

16. (Twice Amended) [Formulation] The formulation according to Claim 3, [characterized in that it comprises] wherein the formulation contains methionine.

17. (Amended) [Formulation] The formulation according to Claim 16, [characterized in that] wherein the methionine is present in a concentration of 0.1 to 4 mmol/l.

18. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it furthermore comprises auxiliaries] further comprising an ingredient for adjusting [the] tonicity.

19. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it furthermore comprises thickeners] comprising a thickener for increasing [the] viscosity.

20. (Twice Amended) [Formulation] The formulation according to Claim 1, [characterized in that it furthermore comprises] further containing at least one physiologically acceptable [preservatives] preservative.

21. (Twice Amended) [Pharmaceutical preparation] A pharmaceutical composition [characterized in that it comprises] comprising a liquid formulation according to Claim 1, and a pharmaceutically acceptable carrier.

22. (Amended) [Pharmaceutical preparation] The pharmaceutical composition according to Claim 21 in a form suitable for oral, parenteral or ophthalmological administration.

23. (Twice Amended) [Pharmaceutical preparation] The pharmaceutical composition according to Claim 21, wherein the composition is in the form of a unit [doses of] dose containing 1 to 25 [MU]  $\times 10^6$  IU of interferon- $\beta$ .

25. (Amended) [Process] A process for [improving the shelf life of] stabilizing a liquid formulation [which comprises] comprising human interferon- $\beta$  as the active ingredient and a buffer for [setting] buffering in a pH range of 5 to 8, [characterized in that a formulation without human serum albumin or/and with one or more amino acids is used, with the proviso that the formulation does not comprise any acidic amino acids, arginine or glycine in amounts of between 0.3 to 5% by weight] said process comprising adding a stabilizing amount of at least one amino acid, provided that the amino acid is not an acidic amino acid, arginine or glycine in an amount of between 0.3 to 5% by weight of the formulation, with the further proviso that human serum albumin is not present in the formulation.

26. (Amended) [Process] The process according to Claim 25, [characterized in that the improved shelf life encompasses improved] wherein the stabilizing comprises increasing at least one of the long-term stability of the in vitro biological [in vitro] activity, [of] the chemical [integrity or/and of] stability and the physical [integrity] stability of the formulation.



Liquid interferon- $\beta$  formulations

## Cross References to Related Applications

### Description

This application is a continuation of PCT/EP98/06065, filed on September 23, 1998.

- 5 The present invention relates to liquid formulations of human interferon- $\beta$ . The formulations are characterized in that they have a pH in the weakly acidic to neutral range between 5 and 8 and that the interferon- $\beta$  is highly stable in solution while retaining the molecular integrity.
- 10

Background  
of the  
Invention  
Field of  
the  
Invention

### Description of Related Art

- Naturally occurring interferons are species-specific proteins, in some cases glycoproteins, which are produced by various cells of the body after induction with viruses, double-stranded RNA, other polynucleotides and antigens. Interferons exhibit a large number of biological activities such as, for example, antiviral, antiproliferative and immunomodulatory properties. At least 3 different types of human interferons have been identified; they are produced by leucocytes, lymphocytes, fibroblasts and cells of the immune system and termed  $\alpha$ -,  $\beta$ - and  $\gamma$ -interferons. Individual types of interferons can furthermore be divided into a large number of subtypes.
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- 25 Native, human interferon- $\beta$  can be prepared commercially by superinduction of human fibroblast cell cultures with poly-IC followed by isolation and purification of the interferon- $\beta$  by chromatographic and electrophoretic techniques. Proteins or polypeptides which exhibit properties similar to those of natural interferon- $\beta$  can also be prepared by recombinant DNA technologies (EP-A-0 028 033; EP-A-0 041 313; EP-A-0 070 906; EP-A-0 287 075; Chernajovsky et al. (1984) DNA 3, 297-308; McCormick et al. (1984) Mol. Cell. Biol. 4, 166-172). Recombinant human interferon- $\beta$  can be produced both in eukaryotic cells (for example CHO cells) and by prokaryotic cells (for example E. coli).
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- 35

The interferons in question are termed interferon- $\beta$ -1a and interferon- $\beta$ -1b respectively. In contrast to interferon- $\beta$ -1b, interferon- $\beta$ -1a is glycosylated (Goodkin (1994) Lancet 344, 1057-1060).

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A prerequisite for the therapeutic use of interferon- $\beta$  is that it is pharmaceutically formulated so that the protein is storage-stable over a prolonged period while retaining the molecular integrity. Interferon- $\beta$  is  
10 unstable and subject to various degradation reactions. These include, in particular, the cleavage of peptide bonds, deamidation, oxidation of the methionin to methionin sulphide, disulphide exchange, and changes in the sugar side chain which even include  
15 deglycosylation.

Owing to the therapeutical benefit of interferons, a series of formulations have been developed in recent years; however, all of them exhibit certain  
20 disadvantages. US Patent No. 4,647,454 (Inter-Yeda Ltd.) describes a formulation of fibroblast interferon- $\beta$  which can be stabilized by addition of polyvinylpyrrolidone (PVP) in the highly acidic range (pH 3.5). Other preferred auxiliaries are mannitol,  
25 human serum albumin and acetate buffers. The formulation is freeze-dried and stored at 4°C.

The Japanese Patent Specification 59 181 224 (Sumitomo Chemical Co.) describes an aqueous solution of  
30 interferons in which polar amino acids such as arginine, asparagine, glutamic acid, glutamine, histidine, lysine, serine and threonine and their sodium salts together with human serum albumin are employed for stabilizing the interferons.

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The international Patent Application WO 95/31213 (Applied Research Systems ARS Holding) describes a liquid formulation for interferon- $\beta$  which is stabilized by addition of a polyol, preferably mannitol, and a

non-reducing sugar or an amino acid. The formulation furthermore comprises a buffer (acetate buffer pH 3.0 to 4.0) and human serum albumin. While formulas with a pH of between 5 and 6 showed an immediate loss in biological activity, the formulas preferred in the patent specification are sufficiently stable at pH values of 3.0 and 4.0. Moreover, the statement regarding stability only refers to the biological activity of the formulation, but not to the molecular integrity of the active ingredient.

The European Patent Application EP 0 215 658 (Cetus Corp.) describes a formulation for recombinant interferon- $\beta$  in which the bioactive compound is dissolved in an aqueous medium at a pH of between 2 and 4 with addition of stabilizers such as human serum albumin or human plasma protein fractions and, if appropriate, dextrose. A further patent application of Cetus Corp. (WO 89/05 158) describes a formulation for interferon- $\beta$  where either glycerin or polyethylene glycopolymers with a mean molecular weight of between 190 to [sic] 1600 daltons are employed as stabilizers at a pH of between 2 and 4. Suitable buffer components which are mentioned are glycine, phosphoric acid and citric acid.

The European Patent Application EP 0 217 645 (Cetus Corp.) describes pharmaceutical preparations with IL-2 or interferon- $\beta$  which are dissolved in an excipient medium at pH 7 to 8 and stabilized with addition of sodium laurate as surfactant. In addition, SDS is also required as further ionic surfactant in order to stabilize these preparations.

The European Patent EP 0 270 799 (Cetus Oncology Corp.) describes a formulation for unglycosylated recombinant interferon- $\beta$  in an inert water-based excipient medium which comprises non-ionic polymeric detergents as stabilizer.

The European Patent Application EP 0 529 300 (Rentschler Biotechnologie GmbH) describes liquid interferon- $\beta$  formulations which comprise a  
5 concentration of 30 or 70 MU/ml recombinant IFN- $\beta$ , sodium chloride and imidazole buffer or sodium phosphate buffer and have a pH of 7.5 (Example 3). These formulations are stable with regard to their biological activity for 4 weeks at a storage  
10 temperature of 25°C. However, the disadvantage of these compositions is that the concentration of interferon- $\beta$  used ( $\geq 30$  MU/ml) is too high for practical applications. Moreover, there is no mention in EP-A-0 529 300 of a reduction in the stability of  
15 liquid interferon- $\beta$  formulations by addition of human serum albumin. In contrast, the addition of human serum albumin is stated as being preferred.

In addition to formulations for interferon- $\beta$ , there are  
20 also described pharmaceutical dosage forms with interferon- $\alpha$ . The European Patent Specification 0 082 481 (Schering Corp.) discloses an aqueous formulation intended for freeze-drying which comprises human serum albumin, in addition to a phosphate buffer  
25 and glycine. Alanine is mentioned as further optional constituent. After reconstitution, the pH of the solution is between 7.0 and 7.4. A further patent application of Schering Corp. (WO 96/11018) discloses stable aqueous solutions in interferon- $\alpha$  which comprise  
30 chelating agents (NaEDTA or citric acid), a surfactant (Polysorbat 80), an isotonizing agent (sodium chloride) and suitable preservatives such as methylparaben, propylparaben, m-cresol or phenol, at a pH of between 4.5 and 7.1. With regard to the biological activity  
35 (standard method of inhibiting the cytopathic effect (CPE) of a virus as described by W.P. Protzman in J. Clinical Microbiology, 1985, 22, pp. 596-599), the aqueous formulations disclosed prove to be stable for 6 months at 25°C (biological activity  $>90\%$  of the

initial activity). However, a determination of the protein content by HPLC carried out in parallel already shows losses in content of between 20.2 (Table 3) or 32.5% (Table 4) after 6 months at 25°C.

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EP-A-0 736 303 (Hoffmann-LaRoche AG) discloses aqueous interferon- $\alpha$  compositions which, in addition to an interferon- $\alpha$ , comprise a non-ionic detergent, a buffer for setting the pH range between 4.5 and 5.5, benzyl  
10 alcohol and, if appropriate, isotonicizing agents. A determination by HPLC identifies a residual content of 84.5% after storage for three months at 25°C and a starting concentration of 18 MU interferon- $\alpha$ 2a, while this value drops to 62.8% when the stabilizer benzyl  
15 alcohol is omitted.

EP-A-0 641 567 (Ciba Geigy AG) describes pharmaceutical compositions which comprise hybrid interferon- $\alpha$  and, as stabilizer, a buffer with a pH of between 3.0 and 5.0.

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US Patent 5,358,708 (Schering Corp.) describes aqueous formulations of interferon- $\alpha$  which comprise methionine, histidine or mixtures of these as stabilizer. After storage of an interferon- $\alpha$  solution at 40°C for two  
25 weeks, it is found that the active ingredient content has decreased by 20%.

The abovementioned formulations for interferons have shortcomings from the present-day view since, for  
30 example, an addition of human serum albumin for stabilizing proteins should be dispensed with, owing to the higher demands for safety from virus contamination by blood donors. Moreover, a number of the above-described formulations require the addition of amino  
35 acids and/or freeze-drying. However, freeze-dried products are complicated to produce and, accordingly, expensive and require an additional pass owing to the necessity of reconstitution, and this additional pass is frequently very difficult to perform, in particular

for patients with a limited power of movement. A series of formulas have unphysiological pH values of below 5.0. While such values are not entirely unusual (see also S. Sweetana and N.J. Aders, Journal of  
5 Pharmaceutical Sciences and Technology, 1996, 50: 330-342), painful irritation must be expected in the case of intramuscular or subcutaneous application. While according to Sweetana and Akers the use of surfactants such as Polysorbat 80 is admissible, a  
10 series of side effects have been described, in particular in new-born and older children, which make the use of such auxiliaries questionable. A review of the toxicity of surfactants can be found in Attwood and Florence (Surfactant Systems, their Chemistry, Pharmacy  
15 and Biology, Chapman and Hall; London, 1983). The pharmacology of Polysorbat 80 is reviewed by R.K. Varma et al. (Arzneim.-Forsch./Drug Res. 35, 1985, 804-808).

On the basis of the abovementioned disadvantages, an  
20 optimal formulation for interferon- $\beta$  should combine the following properties:

- retaining the biological activity over the storage period,
- 25 - retaining the molecular integrity of the active ingredient molecule over the storage period,
- liquid formulation, no expensive freeze-drying and no additional reconstitution,
- no risky auxiliaries such as human serum albumin  
30 or surfactants (detergents),
- pH in the neutral to weakly acidic range.

All requirements are met by the invention, which is  
described in greater detail in the section which  
35 follows.

### Detailed Description of Invention

Surprisingly, a composition of a formula has been found which ensures the molecular integrity of interferon- $\beta$  in liquid form over a prolonged period in a

← Brief Summary of Invention

(insert block from page 7)

physiological pH range of between 5 and 8, preferably between over 5.5 and 8, without having to resort to the auxiliaries of the prior art, which are known as being disadvantageous.

move to page 6

5 A first aspect of the present invention is therefore a liquid pharmaceutical formulation which comprises human interferon- $\beta$  as active ingredient in a concentration of up to 25 MU/ml and a buffer for setting a pH of between  
10 5 and 8, preferably between over 5.5 and 8, is free from human serum albumin and shows a long-term stability of the biological activity (in vitro) of at least 80% of the initial activity after storage for 3 months at 25°C.

15 A further aspect of the invention is a liquid pharmaceutical formulation which comprises human interferon- $\beta$  as active ingredient and a buffer for setting a pH of between 6 and 7.2, is free from human  
20 serum albumin and shows a long-term stability of the biological activity (in vitro) of at least 80% of the initial activity after storage for 3 months at 25°C.

25 Yet a further aspect of the invention is a liquid pharmaceutical formulation which comprises human IFN- $\beta$  as active ingredient, a buffer for setting a pH of between 5 and 8, preferably between over 5.5 and 8, and one or more amino acids and shows a long-term stability of the biological activity (in vitro) of at least 80%  
30 of the initial activity after storage for 3 months at 25°C.

The long-term stability of liquid pharmaceutical formulations was measured at 25°C. The temperature of  
35 25°C was chosen, on the one hand, to cause accelerated degradation reactions, but, on the other hand, to avoid artefacts caused by unduly high temperatures. Suitable analytical methods for determining the stability of interferon- $\beta$  can be found in the reviews by J. Geigert

(J. Parent. Sci. Technol. 43 (1989), 220-224) or M.C. Manning, K. Patel and R.T. Borchardt (Pharm. Res. 6 (1989), 903-918).

- 5 The biological activity after the storage period chosen in each case was measured by the standard method of inhibiting the cytopathic effect of a virus. A detailed description of the test method used can be found in Stewart, W.E. II (1981): The Interferon System (Second, enlarged Edition), Springer-Verlag: Vienna, New York; Grossberg, S.E. et al. (1984), Assay of Interferons. In: Came, P.E., Carter W.A (eds) Interferons and their Applications, Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, pp. 23-43. After storage for three months  
10 at 25°C, a formulation according to the invention exhibits a biological activity of at least 80%, preferably of at least 85%, and especially preferably of at least 90%, of the initial activity.
- 20 After storage for six months at 25°C, a formulation according to the invention preferably has a biological activity of at least 80%, and preferably of at least 85%, of the initial activity.
- 25 Even when stored at higher temperatures, for example 37°C, the formulations according to the invention exhibit a surprisingly high long-term stability of the biological activity. For example after storage for one month at 37°C, a biological activity of at least 70%,  
30 and preferably of at least 80%, of the initial activity is found.

The liquid pharmaceutical formulations according to the invention are preferably free from human serum albumin  
35 and especially preferably - apart from the active ingredient - free from human or animal polypeptides, in particular serum proteins. It is furthermore preferred for the liquid pharmaceutical formulation according to the invention to be free from surfactants, in

particular free from ionic detergents and/or non-ionic surfactants.

The formulations according to the invention comprise,  
5 as active ingredient, an interferon- $\beta$ , that is to say a polypeptide which exhibits biological and/or immunological properties of natural human interferon- $\beta$  and which may be a naturally occurring or recombinant interferon- $\beta$ . The formulation preferably comprises a  
10 glycosylated interferon- $\beta$ , especially preferably a recombinant interferon- $\beta$  from CHO cells. Interferon- $\beta$  species which are most preferably used are those which can be obtained from the cell line BIC 8622 (ECACC 87 04 03 01) and which are described, for example, in  
15 EP-B-0 287 075 and EP-A-0 529 300.

Preferably, the active ingredient is present in the formulations according to the invention in a concentration of up to 25 MU/ml. However, a dosage in  
20 the range of 1 to 25 MU/ml is preferred, in the range of 3 to 20 MU/ml especially preferred and in the range of 3 to 10 MU/ml most preferred. These dosage ranges allow an immediate use without further dilution in conjunction with a particularly good stability at an  
25 elevated temperature.

A further preferred feature of the liquid pharmaceutical formulation according to the invention is that it exhibits a chemical integrity after storage  
30 for 3 months, and preferably 6 months, at 25°C, i.e. that it is stable to peptide cleavage, oxidation and deglycosylation. The chemical integrity is measured by peptide mapping, Western blot and glycosylation analysis. Chemically stable for the purposes of the  
35 present invention are compositions in which the interferon- $\beta$  after formulation retains at least 85%, preferably at least 90%, of the chemical integrity at the storage conditions chosen.

A further preferred feature of the liquid pharmaceutical formulations according to the invention is a physical integrity after storage for 3 months, and preferably 6 months, at 25°C. The physical integrity is  
5 in this case measured by measuring the transmission at 420 nm and by visually observing the solutions. Physically stable are those solutions whose transmission is over 90%, preferably over 93%, at the storage conditions chosen, and where no turbidity can  
10 be determined upon visual observation.

The present invention surprisingly allows liquid formulations of interferon- $\beta$  to be provided which are biologically, chemically and physically stable over a  
15 prolonged period and free from undesired constituents such as, for example, human serum albumin or surfactants. In addition to the active ingredient, the formulations according to the invention comprise a buffer which is preferably present in a concentration  
20 of 10 mmol/l to 1 mol/l, especially preferably in a concentration of 20 mmol/l to 200 mmol/l, for example approximately 50 mmol/l to 100 mmol/l, and which serves to maintain the pH of the formulation in the range of 5 to 8, preferably above 5.5 to 8, more preferably  
25 between 6 and 7.4. A pH range between 6 and 7.2 is especially preferred, and a pH range between 6.2 and 6.8 most preferred, since a particularly high stability while retaining the molecular integrity is achieved here. The buffer is selected from amongst  
30 pharmaceutically acceptable buffers, for example borate, succinate, L-malate, TRIS, salicylate, glycylglycine, triethanolamine, isocitrate, maleate, phosphate, citrate and acetate buffer, or mixtures of these. Phosphate, citrate and acetate buffer or  
35 mixtures of these are preferably used, especially preferably phosphate/citrate buffers.

In addition to the active ingredient and the buffer, the formulation according to the invention can comprise

other physiologically acceptable auxiliaries, for example auxiliaries for adapting tonicity to the tonicity of blood or tissue, for example non-reducing sugars, sugar alcohols such as mannitol, sorbitol, 5 xylitol or glycerin. Moreover, one or more amino acids such as, for example, alanine, arginine, glycine, histidine or/and methionine, may be added to the formulation according to the invention to further increase the chemical stability. Methionine is 10 preferred in this context. The methionine concentration is preferably in the range of 0.1 to 4 mmol/l. A concentration of 2 mmol/l is especially preferred. Moreover, the composition may comprise thickeners for increasing the viscosity, for example for ophthalmo- 15 logical purposes. Examples of suitable thickeners are ophthalmologically suitable polymers, for example Carbopol, methylcellulose, carboxymethylcellulose etc.

Moreover, the composition according to the invention 20 may also comprise preservatives. For ophthalmological purposes, for example, thiomersalate may be employed in an amount of 0.001 to 0.004% (weight/volume).

The invention furthermore relates to pharmaceutical 25 preparations which comprise a liquid interferon- $\beta$ -comprising formulation as described above. These pharmaceutical preparations are particularly suitable for oral, parenteral or ophthalmological application. The formulations preferably exist in unit doses of 1 to 30 25 MU IFN- $\beta$ . The invention furthermore relates to a process for the preparation of such pharmaceutical preparation, in which a formulation according to the invention and, if appropriate, other pharmaceutical formulation auxiliaries which are necessary are 35 prepared and formulated as a suitable dosage form.

The formulation according to the invention can be stored in suitable, washed and sterilized glass vials (hydrolytic class 1) with pharmaceutically acceptable

rubber stoppers.

Moreover, formulations according to the invention can also be packaged aseptically in ready-to-use syringes or else in carpules for use in self-injection systems, and employed thus. While this is not preferred, the aqueous solutions may be freeze-dried by addition of other auxiliaries known to the skilled worker, and, after reconstitution, are available in liquid form.

10

Using suitable preservatives, it is possible to prepare liquid multidose forms and eye-drop solutions and solutions for dropwise oral application.

15 The auxiliaries additionally required for preparing the relevant dosage forms are known to the skilled worker.

Finally, the invention relates to a process for improving the shelf life of a liquid formulation which comprises human interferon- $\beta$  as active ingredient and a buffer for setting a pH of 5 to 8, preferably of above 5.5 to 8, characterized in that a formulation without human serum albumin or/and with one or more amino acids is used. The improvement in shelf life encompasses improved long-term stability of the biological activity (in vitro), of the chemical integrity or/and of the physical integrity as indicated hereinabove.

20 The invention is furthermore illustrated by the examples which follow.

#### **Examples**

An interferon- $\beta$  obtained from CHO cells was used in all the examples.

35

#### 1. Long-term stability of liquid interferon- $\beta$ formulations at 25°C

The following formulations were tested:

- Formulation 1: 50 mmol/l sodium citrate pH 5.0
- Formulation 2: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 15 mg/ml human serum albumin, 2 mmol/l methionine, 50 mg/ml glycerin
- Formulation 3: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 50 mg/ml glycerin, 2 mmol/l methionine
- Formulation 4: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 2 mmol/l methionine
- Formulation 5: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0
- Formulation 17: 70 mmol/l sodium citrate, 50 mmol/l sodium phosphate, 2 mmol/l methionine, pH 6.5

The formulations were diluted to a content of approx. 10 to 15 MU/ml (that is to say 10 to 15  $\times 10^6$  IU/ml).

With the exception of formulation 17 (see below), the formulations were stored at 25°C for the period indicated in hydrolytic class 1 glass vials (DIN 2R vials) which were sealed with commercially available chlorobuthyl rubber stoppers. The biological activity (in vitro) was determined as described by Stewart, W.E. II (1981): The Interferon System (Second, enlarged edition) Springer-Verlag: Vienna, New York; Grossberg, S.E. et al. (1984) Assay of Interferons. In: Came, P.E., Carter W.A. (eds.) Interferons and their Applications, Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, pp. 23-43.

The results are shown in Tables 1 to 5. "% (ref.)" indicates the biological activity based on the biological activity of a reference sample which had been stored at -20°C for the period indicated. "% (0mo)" is the percentage of biological activity based on the initial value at 0 months.

Table 1 (Formulation 1):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	11.0	11.0	100	100
1	10.0	9.8	98	89
2	9.7	11.0	113	100
3	10.0	10.6	106	96
4	10.3	9.5	92	86
5	9.5	9.7	102	88
6	10.5	10.2	97	93

5 Table 2 (Formulation 2):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	13.9	13.9	100	100
1	14.0	11.9	85	86
2	13.0	11.6	89	83
3	13.1	9.6	73	69
4	12.5	8.8	70	63
5	11.0	8.2	75	59
6	13.3	8.4	63	60

Table 3 (Formulation 3):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	12.5	12.5	100	100
1	9.4	10.0	106	80
2	8.3	11.5	139	92
3	7.8	11.8	151	94.4
4	6.8	10.3	151	82.4
5	6.6	11.2	170	89.6

6	7.8	13.4	172	107.2
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Table 4 (Formulation 4):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	11.4	11.4	100	100
1	10.5	10.2	97	89
2	11.9	11.1	93	97
3	10.8	10.0	93	88
4	10.4	9.3	89	82
5	11.6	8.4	72	74
6	12.4	9.5	77	83

5 Table 5 (Formulation 5):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	11.3	11.3	100	100
1	11.0	9.7	88	86
2	11.7	10.1	86	89
3	11.1	10.2	92	90
4	11.3	10.2	90	90
5	12.0	9.2	77	81
6	11.0	9.7	88	86

10 It can be seen from the above tables that formulations which do not contain human serum albumin (Formulations 1, 3, 4, 5) surprisingly exhibit a better stability than a formulation which comprises human serum albumin (Formulation 2).

15 In Formulation 17 (see above), an interferon solution without human serum albumin was brought to an activity of 6 MU/0.5 ml under aseptic conditions. The colourless, clear solution was subsequently filter-sterilized, and 0.5-ml aliquots were filled into pre-

sterilized disposable syringes and sealed. The ready-to-use syringes were stored at 25°C and examined for clarity, pH and biological activity. The following results were obtained:

5

Storage in months	pH	Clarity[%]	MU/syringe		Recovery (25°C)	
			-20°C	25°C	% (ref.)	% (0mo.)
0	6.5	99.5	6.3	6.3	100	100
3	6.5	99.1	5.6	6.1	108	97

## 2. Long-term stability of liquid IFN-β formulations at 37°C

10 The following formulations in ready-to-use syringes were tested:

Formulation 6: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 2 mmol/l methionine

15

Formulation 7: 50 mmol/l sodium citrate pH 5.0, 18 mg/ml glycerin, 2 mmol/l methionine

Formulation 8: 50 mmol/l sodium citrate pH 5.0, 18 mg/ml glycerin, 15 mg/ml human serum albumin, 2 mmol/l methionine

20

Formulation 9: 50 mmol/l sodium citrate pH 6.0, 18 mg/ml glycerin, 2 mmol/l methionine

Formulation 10: 50 mmol/l sodium citrate pH 6.5, 18 mg/ml glycerin, 2 mmol/l methionine

25

The formulations were tested in dosage strengths of 3 MU per 0.5 ml (dosage strength 3), 6 MU per 0.5 ml (dosage strength 6) and 12 MU per ml (dosage strength 12).

30

The results are shown in Table 6 which follows.

Table 6

Storage in months	Dosage strength 3					Dosage strength 6					Dosage strength 12				
	Formulation					Formulation					Formulation				
0	6	7	8	9	10	6	7	8	9	10	6	7	8	9	10
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	71	80	61	74	69	72	85	63	86	84	87	88	71	76	84
2	51	82	33	74	85	61	81	43	80	76	69	88	48	77	81
3	44	76	23	63	65	48	64	36	73	69	66	72	35	80	81
4	33	51	16	61	61	46	65	26	84	-	-	64	24	78	79

The results of Table 6 show that, surprisingly, the formulations according to the invention without human serum albumin exhibit an improved stability at 37°C.

5    3. Chemical stability at 25°C

To examine the chemical stability of liquid formulations of IFN- $\beta$ , 7 batches were formulated and stored at 25°C. After 3 and 6 months, the protein was  
10 characterized by means of Lys-C mapping and complete carbohydrate analysis. The formation of methionine sulfoxide and the desialylation was checked particularly carefully.

15 In addition to Formulation 10 (see above), the following formulations were tested:

Formulation 11: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate, 2 mmol/l methionine  
20 pH 7.0 to 7.2

Formulation 12: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0 to 7.2

Formulation 13: 50 mmol/l sodium citrate, 18 mg/ml glycerin, 2 mmol/l methionine, pH 5.0  
25 to 5.2

Formulation 14: 50 mmol/l sodium citrate, 18 mg/ml glycerin, pH 5.0 to 5.2

Formulation 15: 50 mmol/l sodium citrate, 15 mg/ml human serum albumin (medical grade),  
30 18 mg/ml glycerin, 2 mmol/l methionine, pH 5.0 to 5.2

Formulation 16: 50 mmol/l sodium citrate, 15 mg/ml human serum albumin (medical grade),  
35 18 mg/ml glycerin, pH 5.0 to 5.2 (comparison)

In all batches, the IFN- $\beta$  content was between 10 and 11 MU/ml.

### Testing procedure

To carry out the analyses, the samples had to be concentrated. Moreover, the human serum albumin had to be removed in the case of batches 15 and 16. This is why the batches were passed over an anti- $\beta$  chromatography column. The initial volume per batch was 32 ml. Batches 13 to 16 were neutralized prior to anti- $\beta$  chromatography by addition of 2.1 ml of 0.4 mol/l  $\text{Na}_2\text{HPO}_4$  and 2.1 ml of 0.4 mol/l  $\text{Na}_3\text{PO}_4$ .

For the immunoadsorption of interferon- $\beta$  on a monoclonal antibody against interferon- $\beta$  (BO2 sepharose 6B, crosslinked by Celltech), a C10 chromatography column (Pharmacia) was packed with 5 ml of BO2 sepharose and washed 3 times with in each case 5-10 gel volumes of PBS, 0.1 mol/l sodium phosphate pH 2.0 and PBS/1 mol/l KCl at a linear flow rate of 1.0 cm/min.

Approximately 32 ml of the interferon/HSA-containing solution was applied at a linear flow rate of 0.5 cm/min.

Washing was effected with 10 gel volumes of PBS/1 mol/l KCl with a linear flow rate of 1 cm/min until the OD had dropped to baseline. Elution was done with approximately 1-2 gel volumes of 0.1 mol/l sodium phosphate pH 2.0 at a linear flow rate of 1 cm/min. Interferon- $\beta$  is obtained as single peak in high purity. This eluate is suitable for the subsequent protein characterization.

### Analytical procedure

35

#### 1. Lys-C mapping

Using the *Achromobacter* (AP) enzyme endoproteinase Lys-

C, interferon- $\beta$  is cleaved under reducing conditions on the C-terminal end of lysin to give 12 peptides.

50  $\mu$ l of eluate from the anti- $\beta$  chromatography  
5 (12.5-50  $\mu$ g of interferon- $\beta$ ) were placed into an Eppendorf reaction vessel, and 5  $\mu$ l of 2 mol/l TRIS were added. Wako endoproteinase was added in an enzyme/substrate ratio of 1:10 (endoproteinase Lys-C solution in 50 mmol/l TRIS/HCl, pH 9.0). The solution  
10 was mixed and incubated for 2 hours at 30°C. Then, 5  $\mu$ l of 0.1 mol/l DTT were added to the batch.

The peptides were separated on a reversed-phase column (Vydac C18, 300 Å, 5  $\mu$ m, 2.1 mm) on an HPLC system HP  
15 1090 M series with diode array detector at 214 nm, for which purpose a gradient of A: 0.1% (v/v) TFA and B: 0.1% (v/v) TFA/70% (v/v) acetonitrile was used. The peptides were numbered consecutively in the sequence of their retention times and are allocated to the  
20 following sequences:

SEQ. ID. No.	Peptide	Position	Sequence
1	AP1	109-115	EDFTRGK
2	AP2	100-105	TVLEEK
3	AP3	46-52	QLQQFQK
4	AP4 (ox)	116-123	LM (ox) SSLHLK
5	AP4	116-123	LMSSLHLK
6	AP6 (ox)	35-45	DRM (ox) NFDIPEEIK
7	AP5	124-134	RYYGRILHYLK
8	AP6	34-45	DRMNFDIPEEIK
9	AP7	20-33	LLWQLNGRLEYCLK
10	AP8 (ox)	1-19	M (ox) SYNLLGFLQRSSNFQCQK
11	AP8	1-19	MSYNLLGFLQRSSNFQCQK
12	AP9	137-166	EYSHCAWTIVRVEILRNFYFINRLTG YLRN
13	AP10 (ox)	53-99	EDAALTIYEM (ox) LQNIFAIFRQDS SSTGWNETIVENLLANVYHQINHLK
14	AP10	53-99	EDAALTIYEMLQNIFAIFRQDSSSTG

			WNETIVENLLANVYHQINHLK
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References:

- Utsumi et al. (1989). Characterization of four different mammalian-cell-derived recombinant human interferon- $\beta$ 1. Eur. J. Biochem. 181, 545-553.
- 5 Utsumi et al. (1988): Structural characterization of fibroblast human interferon- $\beta$ 1. J. Interferon Res. 8, 375-384
- Allen, G. (1981): Laboratory techniques in biochemistry and molecular biology. Sequencing of proteins and
- 10 peptides. Elsevier Verlag.
- Castagnola et al. (1988): HPLC in Protein sequence determinations. J. Chromatography 440, 213-251.

In the peptides marked (ox), the amino acid methionine

15 is in the form of methionine sulphoxide. The quantification is based on determining the proportion of the peak area of the oxidized peptide relative to the total area of intact peptide and oxidized peptide. The proportions of oxidized methionines are very low in

20 fresh interferon- $\beta$  preparations. However, the proportion increases more or less drastically during storage, depending on the storage conditions (buffer, pH, temperature etc.). This change is undesired since it contributes to the instability of the interferon- $\beta$

25 molecule or can significantly affect the in-vivo properties.

The proportion of the oxidized peptides AP4(ox), AP6(ox), AP8(ox) and AP10(ox) is thus an important

30 criterion for assessing the chemical integrity of the interferon- $\beta$  molecule in a liquid formulation.

## 2. Carbohydrate determination

35 In the first step, the oligosaccharides were separated from the polypeptide and demineralized.

Approximately 0.7 ml of the eluate of the anti- $\beta$  chromatography were dialysed for 16-20 hours at room temperature in a dialysis tubing (diameter 6 mm, Sigma No. D-9277) against 500 ml of dialysis buffer (0.05 mol/l sodium phosphate, 0.10 mol/l NaCl, pH 7.25), with gentle stirring. Then, the tubing was cut open at one end and the contents squeezed into a Eppendorf reaction vessel. After dialysis, the sample volume was 1 ml.

20  $\mu$ l of Tween 20 (10% strength) and 15  $\mu$ l of N-glycosidase F solution (Boehringer Mannheim) were pipetted to the dialysed sample. This mixture was incubated for 24 hours at 37°C. After the incubation had ended, the mixture was centrifuged for 10 minutes at 10,000 rpm, filtered through a 0.45  $\mu$ m filter and subsequently chromatographed and fractioned over a desalting column (HR 10/10 Pharmacia No. 17-0591-01) with an isocratic gradient (eluent A: distilled water) at a flow rate of 1.0 ml/min. The free oligosaccharides were detected at 206 nm.

In the second step, the oligosaccharides which had been liberated were separated by an ion exchanger as a function of the number of the sialic acid residues.

The oligosaccharides contained in the eluate of the desalted column, approx. 2 ml, were bound to an anion exchanger (Mono Q HR 5/5, Pharmacia No. 17-0546-01). The asialo forms are in the eluate. With the aid of a shallow NaCl gradient, monosialo, disialo and trisialo forms eluted distinctly separately one after the other in the sequence indicated.

Eluent A: Milli-Q water  
Eluent B: 0.10 mol/l NaCl

Gradient

0 min	100% A	0% B
5 min	100% A	0% B
25 min	33% A	67% B
26 min	100% A	0% B

Flow rate: 0.75 ml/min

Chromatography time: 26 min (with regeneration 36 min)

5 Detection: UV 206 nm

The individual oligosaccharide fractions were detected by means of a UV detector at 206 nm. The quantitative calculation was done by integrating the areas of the individual peaks.

The oligosaccharide fractions monosialo, disialo and trisialo were subsequently passed over a desalting column as described above.

15 In the third step, the charged oligosaccharides are converted into neutral oligosaccharides by hydrolytically eliminating the terminal sialic acid residues under acidic pH conditions.

20 To this end, approx. 15 µl of each oligosaccharide fraction plus 15 µl of Milli Q water were placed into a micro-test tube, and 30 µl of 10 mmol/l H<sub>2</sub>SO<sub>4</sub> were added. The mixture was then heated for 90 minutes at 25 80°C.

Then, the batch was centrifuged for 1 minute at 5000 rpm and pipetted into a minivial. The carbohydrates, which are now neutral, are bound at alkaline pH to weak anions and on an anion-exchanger column (CarboPac PA1 (4x250 mm) P/N 35391, Dionex). Elution is done with a gradient of

Eluent A: NaOH 0.16 mol/l

Eluent B: NaOH 0.16 mol/l sodium acetate 0.10 mol/l

Eluent C: NaOH 0.16 mol/l sodium acetate 0.75 mol/l

Gradient:

5

0 min	95% A	5% B	0% C
2.0 min	95% A	5% B	0% C
3.0 min	85% A	15% B	0% C
4.0 min	85% A	15% B	0% C
28.0 min	37% A	63% B	0% C
28.1 min	90% A	0% B	10% C
45.0 min	20% A	0% B	80% C
45.1 min	95% A	5% B	0% C
50.0 min	95% A	5% B	0% C

Flow rate: 1.0 ml/min

Chromatography time: 50 min

Detection: PAD

10

PAD (pulsed amperometric detection) was used to determine the oligosaccharides. The oligosaccharide molecule is electrochemically oxidized, and the current thus formed measured. PAD is distinguished by a high sensitivity, so that a detection in the ng range presents no difficulty. The output signal in the detector (in mV) is directly proportional to the amount of carbohydrate. Quantification is done by integrating the peak areas.

20

Between the deglycosylation and the analysis, the samples were subjected to intermediate storage at -20°C.

25 References:

Townsend (1988): High-performance anion-exchange chromatography of oligosaccharides. Analytical Biochemistry 174, 459-470.

## Results

### 1. Lys-C mapping

- 5 The Lys-C mapping of batches 11 to 16 showed no difference to the initial value with regard to retention time and qualitative determination of the peptides.
- 10 The determination of the methionine sulfoxide content during liquid storage revealed the results shown in Tables 7 (3 months' storage) and 8 (6 months' storage).

Table 7

15

Name	AP4ox content	AP6ox content	AP8ox content	AP10ox content
to value	< 5%	7.6%	n.d.	n.d.
Formulation 11	7.9%	10.5%	n.d.	n.d.
Formulation 12	< 5%	11.6%	n.d.	n.d.
Formulation 13	< 5%	7.3%	n.d.	n.d.
Formulation 14	< 5%	9.4%	n.d.	n.d.
Formulation 15	< 5%	8.6%	n.d.	n.d.
Formulation 16	< 5%	10.8%	n.d.	n.d.

(n.d. = not detectable)

Table 8

Name	AP4ox content	AP6ox content	AP8ox content	AP10ox content
to value	< 5%	7.6%	n.d.	n.d.
Formulation 10	7.6%	8.9%	n.d.	n.d.
Formulation 11	7.7%	9.5%	n.d.	n.d.
Formulation 12	12.0%	13.7%	n.d.	n.d.
Formulation 13	7.4%	8.7%	n.d.	n.d.
Formulation 14	13.7%	15.7%	n.d.	n.d.
Formulation 15	7.4%	7.9%	n.d.	n.d.
Formulation 16	18.0%	17.6%	n.d.	n.d.

Table 7 reveals that the methionine-containing batches 13 and 15 show a lower methionine sulphoxide content upon three months' storage in comparison with methionine-free batches. After storage for six months, the affect of the added methionine in batches 11, 13 and 15 is more pronounced. Only a very small increase in the methionine sulphoxide content can be detected in these batches. In the methionine-free batches, the methionine sulphoxide content increases slightly more, but the total of all oxidized methionine contents amounts to less than 10% of the total methionine content.

## 2. Carbohydrate determination

The results of the carbohydrate determination after storage for three or 6 months are shown in Tables 9a, 9b, 10a, 10b, 11a and 11b.

Interferon- $\beta$ -1a has a carbohydrate structure on its amino acid chain which is composed of a defined sequence of monosaccharides. Depending on the type of branching, these structures are termed biantennary (2 arms), triantennary (3 arms) and tetraantennary (4 arms).

The carbohydrate structure is composed of the monosaccharides mannose, fucose, N-acetylglucosamine, galactose and sialic acid.

In this context, the sialic acid is special in several respects:

- It is the only monosaccharide with a charged group (carboxyl group).
- It always occurs at the terminus of the carbohydrate chain.
- It can be eliminated enzymatically or

hydrolytically considerably more readily than the remaining monosaccharides.

- While the structure of the neutral carbohydrate chain is highly constant, the sialic acid moiety varies greatly depending, inter alia, on the cell culture and the purification method of the interferon.

References:

- 10 Kagawa et al., J. Biol. Chem. 263 (1988), 17508-17515; EP-A-0 529 300.

The sialostatus (percentage of individual sialo structures) after three months' storage (Table 9a) or six months' storage (Table 9b) was investigated. A carbohydrate structure which does not contain a terminal sialic acid is termed asialo. A carbohydrate structure which contains a terminal sialic acid is termed monosialo. A carbohydrate structure which contains two terminal sialic acids is termed disialo. A carbohydrate structure which contains three terminal sialic acids is termed trisialo.

Furthermore, the antennarity (percentage of individual branching types) was determined after three months' storage (Table 10a) and after six months' storage (Table 10b). A carbohydrate structure with one branching and thus two terminal galactoses is termed biantennary. It can have zero to two terminal sialic acids. A carbohydrate structure with two branchings and thus three terminal galactoses is termed triantennary. It can have zero to three terminal sialic acids.

The degree of sialylation (percentage occupation of terminal galactose residues with sialic acid) after three months' storage (Table 11a) and six months' storage (Table 11b) was also investigated.

It can be seen from the results that storage at pH 5 causes a slight, but reproducible, desialylation. Storage at pH 7 has no effect on the degree of sialylation.

5

The afuco content specified in batches 15 and 16 is probably due to foreign proteins from the added human serum albumin, which were not quantitatively removed by anti- $\beta$  chromatography.

10

As regards the antennarity, liquid storage has no measureable effect.

Table 9a

15

Name	Asialo	Monoasialo	Disialo	Trisialo
to value	< 3	13.4	73.4	12.1
Formulation 11	< 3	14.0	74.1	11.9
Formulation 12	< 3	12.6	74.9	11.6
Formulation 13	< 3	16.5	70.4	12.0
Formulation 14	< 3	16.6	71.1	11.1
Formulation 15	< 3	15.8	70.0	13.0
Formulation 16	< 3	15.1	72.0	11.9

Table 9b

Name	Asialo	Monoasialo	Disialo	Trisialo
to value	< 3	13.4	73.4	12.1
Formulation 10	< 3	13.9	70.2	15.3
Formulation 11	< 3	14.5	73.9	11.6
Formulation 12	< 3	14.0	72.4	13.6
Formulation 13	< 3	18.6	68.9	11.7
Formulation 14	< 3	19.0	69.4	10.7
Formulation 15	< 3	17.0	71.0	11.3
Formulation 16	< 3	16.1	71.5	12.4

Table 10a

Name	Biantennary	Triantennary 1 → 6	Triantennary + 1 repeat
to value	74.4	18.1	3.7
Formulation 11	72.9	18.7	3.7
Formulation 12	76.9	17.0	2.7
Formulation 13	74.7	18.0	3.1
Formulation 14	75.9	17.3	2.9
Formulation 15	76.2 (incl. 5% afuco)	18.0	3.3
Formulation 16	76.9 (incl. 5% afuco)	17.8	3.0

Table 10b

5

Name	Biantennary	Triantennary 1 → 6	Triantennary + 1 repeat
to value	74.4	18.1	3.7
Formulation 10	71.4	19.3	4.0
Formulation 11	73.0	18.7	3.3
Formulation 12	72.3	19.7	3.4
Formulation 13	72.4	19.2	3.4
Formulation 14	74.2	18.7	3.2
Formulation 15	73.0	18.7	2.8
Formulation 16	74.3 (incl. 4% afuco)	19.7	3.2

Table 11a

Name	Degree of sialylation
to value	88.3
Formulation 11	87.0
Formulation 12	88.2
Formulation 13	85.8
Formulation 14	85.8
Formulation 15	86.6
Formulation 16	86.9

Table [11a] 11b

5

Name	Degree of sialylation
to value	88.3
Formulation 10	87.5
Formulation 11	86.6
Formulation 12	87.7
Formulation 13	84.1
Formulation 14	84.3
Formulation 15	85.7
Formulation 16	86.5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Dr.Rentschler Biotechnologie GmbH  
(B) STREET: Erwin-Rentschler-Str. 21  
(C) TOWN: Laupheim  
(E) COUNTRY: Germany  
(F) POSTCODE: D-88471

(ii) TITLE OF THE INVENTION: Liquid interferon- $\beta$   
formulations

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER-READABLE VERSION:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM/PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: Patent In Release #1.0, Version  
#1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 aminoacids  
(B) TYPE: aminoacid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 109-115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Asp Phe Thr Arg Gly Lys  
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 aminoacids  
(B) TYPE: aminoacid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

5 (B) MAP POSITION: 100-105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Thr Val Leu Glu Glu Lys  
1 5

10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 7 aminoacids  
(B) TYPE: aminoacid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 46-52

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

25

Gln Leu Gln Gln Phe Gln Lys  
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 aminoacids  
(B) TYPE: aminoacid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 116-123

40

(ix) FEATURE:

(A) NAME/KEY: Modified-site  
(B) POSITION: 2  
(C) OTHER INFORMATION: /product= "Xaa =  
45 Met(oxidized)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

**Leu Xaa Ser Ser Leu His Leu Lys**  
**1 5**

5 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 aminoacids
- (B) TYPE: aminoacid
- 10 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (viii) POSITION IN THE GENOME:

(B) MAP POSITION: 116-123

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

**Leu Met Ser Ser Leu His Leu Lys**  
**1 5**

20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 12 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 34-45

35 (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) POSITION: 3
- (C) OTHER INFORMATION: /product= "Xaa =  
Met(oxidized)"

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

**Asp Arg Xaa Asn Phe Asp Ile Pro Glu Glu Ile Lys**  
**1 5 10**

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 124-134

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

**Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys**  
**1 5 10**

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 34-45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

**Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys**  
**1 5 10**

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 20-33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu L u Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu Lys  
1 5 10

(2) INFORMATION FOR SEQ ID NO: 10:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 aminoacids

(B) TYPE: aminoacid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

15

(B) MAP POSITION: 1-19

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) POSITION: 1

20

(C) OTHER INFORMATION: //product= "Xaa =  
Met(oxidized)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

25

Xaa Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln  
1 5 10 15  
Cys Gln Lys

(2) INFORMATION FOR SEQ ID NO: 11:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 aminoacids

(B) TYPE: aminoacid

(C) STRANDEDNESS: single

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

40

(B) MAP POSITION: 1-19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln  
1 5 10 15  
Cys Gln Lys

45

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 137-166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Glu Tyr Ser His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg  
 1 5 10 15  
 Asn Phe Tyr Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn  
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 53-99

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) POSITION: 10
- (C) OTHER INFORMATION: /product= "Xaa = Met(oxidized)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Glu Asp Ala Ala Leu Thr Ile Tyr Glu Xaa Leu Gln Asn Ile Phe Ala  
 1 5 10 15  
 Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val  
 20 25 30  
 Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys  
 35 40 45

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

5

- (A) LENGTH: 47 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 53-99

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

15

Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala  
1 5 10 15  
Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val  
20 25 30  
Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys  
35 40 45

**Patent Claims**

1. Liquid formulation which comprises human  
interferon- $\beta$  as active ingredient in a  
5 concentration of up to 25 MU/ml and a buffer for  
setting a pH of 5 to 8, is free from human serum  
albumin and shows a long-term stability of the  
biological activity (in vitro) of at least 80% of  
the initial activity after storage for 3 months at  
10 25°C.
2. Liquid formulation which comprises human  
interferon- $\beta$  as active ingredient and a buffer for  
setting a pH of 6 to 7.2, is free from human serum  
15 albumin and shows a long-term stability of the  
biological activity (in vitro) of at least 80% of  
the initial activity after storage for 3 months at  
25°C.
- 20 3. Liquid formulation which comprises human  
interferon- $\beta$  as active ingredient, a buffer for  
setting a pH of 5 to 8, and one or more amino  
acids and shows a long-term stability of the  
biological activity (in vitro) of at least 80% of  
25 the initial activity after storage for 3 months at  
25°C.
4. Formulation according to Claim 1,  
**characterized in that**  
30 it comprises a glycosylated interferon- $\beta$ .
5. Formulation according to Claim 2,  
**characterized in that**  
the interferon- $\beta$  originates from CHO cells.  
35
6. Formulation according to any of Claims 1 to 5,  
**characterized in that**

it comprises the buffer in a concentration of 10 mmol/l to 1 mol/l.

7. Formulation according to any of Claims 1 to 6,  
5 **characterized in that**  
it comprises a buffer selected from the group consisting of phosphate, citrate and acetate buffers and mixtures of these.
- 10 8. Formulation according to Claim 7,  
**characterized in that**  
it comprises a phosphate/citrate buffer.
- 15 9. Formulation according to any of Claims 1 and 3 to 8,  
**characterized in that**  
it has a pH between 6 and 7.2.
- 20 10. Formulation according to Claim 3,  
**characterized in that**  
it is free from human serum albumin.
- 25 11. Formulation according to any of Claims 1 to 10,  
**characterized in that,**  
apart from the active ingredient, it is free from human or animal polypeptides.
- 30 12. Formulation according to any of Claims 1 to 11,  
**characterized in that**  
it is free from surfactants.
- 35 13. Formulation according to any of Claims 1 to 12,  
**characterized in that**  
it exhibits a chemical integrity after storage for 6 months at 25°C.
14. Formulation according to any of Claims 1 to 13,  
**characterized in that**

it exhibits a physical integrity after storage for 6 months at 25°C.

15. Formulation according to any of Claims 1, 2 and 4  
5 to 14,  
**characterized in that**  
it furthermore comprises one or more amino acids.
16. Formulation according to Claim 3 or 15,  
10 **characterized in that**  
it comprises methionine.
17. Formulation according to Claim 16,  
**characterized in that**  
15 the methionine is present in a concentration of 0.1 to 4 mmol/l.
18. Formulation according to any of Claims 1 to 17,  
**characterized in that**  
20 it furthermore comprises auxiliaries for adjusting the tonicity.
19. Formulation according to any of Claims 1 to 18,  
**characterized in that**  
25 it furthermore comprises thickeners for increasing the viscosity
20. Formulation according to any of Claims 1 to 19,  
**characterized in that**  
30 it furthermore comprises physiologically acceptable preservatives.
21. Pharmaceutical preparation,  
**characterized in that**  
35 it comprises a liquid formulation according to any of Claims 1 to 20.

22. Pharmaceutical preparation according to Claim 21 for oral, parenteral or ophthalmological administration.
- 5 23. Pharmaceutical preparation according to Claim 21 or 22 with unit doses of 1 to 25 MU.
24. Process for the preparation of a pharmaceutical preparation according to any of Claims 21 to 23,  
10 **characterized in that**  
a formulation according to any of Claims 1 to 20 and, if appropriate, other pharmaceutical formulation auxiliaries which are necessary is prepared and formulated as a suitable dosage form.
- 15 25. Process for improving the shelf life of a liquid formulation which comprises human interferon- $\beta$  as active ingredient and a buffer for setting a pH of 5 to 8,  
20 **characterized in that**  
a formulation without human serum albumin or/and with one or more amino acids is used.
- 25 26. Process according to Claim 25,  
**characterized in that**  
the improved shelf life encompasses improved long-term stability of the biological activity (in vitro), of the chemical integrity or/and of the physical integrity.

## Abstract

The present invention relates to liquid formulations of human interferon- $\beta$ . The formulations are characterized in that they have a buffer with a pH in the weakly acidic to neutral range of between 5 and 8, preferably between over 5.5 and 8, and that they exhibit high stability of the interferon- $\beta$  in solution while retaining the molecular integrity.

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PCT/EP98/06065

17150P WO

Enclosure

5

**Patent Claims**

1. Liquid formulation which comprises human  
interferon- $\beta$  as active ingredient in a  
concentration of up to 25 MU/ml and a buffer for  
10 setting a pH of 5 to 8, is free from human serum  
albumin and after storage for 3 months at 25°C  
shows a long-term stability of the biological in  
vitro activity of at least 80% of the initial  
activity, with the proviso that the formulation  
15 does not comprise any acidic amino acids, arginine  
or glycine in amounts of between 0.3 and 5% by  
weight.
3. Liquid formulation which comprises human  
20 inferferon- $\beta$  as active ingredient, a buffer for  
setting a pH of 5 to 8, and one or more amino  
acids and shows after storage for 3 months at 25°C  
a long-term stability of the biological in vitro  
activity of at least 80% of the initial activity,  
25 with the proviso that the formulation does not  
comprise any acidic amino acids, arginine or  
glycine in amounts of between 0.3 and 5% by  
weight.

23. Pharmaceutical preparation according to Claim 21 or 22 in the form of unit doses of 1 to 25 MU.
- 5 25. Process for improving the shelf life of a liquid formulation which comprises human interferon- $\beta$  as active ingredient and a buffer for setting a pH of 5 to 8,  
**characterized in that**  
a formulation without human serum albumin or/and  
10 with one or more amino acids is used, with the proviso that the formulation does not comprise any acidic amino acids, arginine or glycine in amounts of between 0.3 to 5% by weight.
- 15 26. Process according to Claim 25,  
**characterized in that**  
the improved shelf life encompasses improved long-term stability of the biological in vitro  
activity, of the chemical integrity or/and of the  
20 physical integrity.

vo - October 5, 1999

New Claim 2

- 5    2.    Liquid formulation according to Claim 1,  
         **characterized in that**  
         it comprises a buffer for setting a pH of 6 to  
         7.2.